Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US2005/011441

International filing date: 04 April 2005 (04.04.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/562,119
Filing date: 14 April 2004 (14.04.2004)

Date of receipt at the International Bureau: 10 July 2007 (10.07.2007)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

1630904

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

July 03, 2007

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/562,119*
FILING DATE: *April 14, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/11441*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US60/562,119*

UNITED STATES PATENT AND TRADEMARK OFFICE · DEPARTMENT OF COMMERCE

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/16 (08-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 981814271 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
John M. Heinz G.	Cassady Floss	Columbus, OH Bellevue, WA

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

MAYTANSINOID ANALOGS AS IMPROVED ANTITUMOR AGENTS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ Customer Number: 24024

OR

☐ Firm or Individual Name					
Address					
Address					
City		State		Zip	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 20 ☐ CD(s), Number _____

☐ Drawing(s) *Number of Sheets* _____ ☑ Other (specify) Return receipt postcard

☐ Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☑ A check or money order is enclosed to cover the filing fees.

☐ The Director is herby authorized to charge filing fees or credit any overpayment to Deposit Account Number: _____

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($)

$ 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Sean Myers-Payne

Date April 14, 2004

REGISTRATION NO. 42,920 *(if appropriate)*

Docket Number: 22727/04241

TYPED or PRINTED NAME Sean C. Myers-Payne

TELEPHONE (614) 621-7754

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PTO/SB/17 (10-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	To Be Determined
Filing Date	April 14, 2004
First Named Inventor	John M. Cassady
Examiner Name	To Be Determined
Art Unit	To Be Determined
Attorney Docket No.	22727/04241

METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☐ Deposit Account:

Deposit Account Number

Deposit Account Name

The Director is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☐ Credit any overpayments

☐ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=
Independent Claims		- 3** =		X	=
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY

(Complete *(if applicable)*)

Name *(Print/Type)*	Sean C. Myers-Payne	Registration No. *(Attorney/Agent)*	42,920	Telephone	(614) 621-7754
Signature	Sean Myers Payne			Date	April 14, 2004

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PROVISIONAL PATENT APPLICATION

FOR

MAYTANSINOID ANALOGS AS ANTITUMOR AGENTS

BY

JOHN M. CASSADY

HEINZ G. FLOSS

DESCRIPTION OF THE INVENTION

Field of the Invention

[001] The present invention relates to ansamycin analogs, including maytansinoid analogs, and their use in treating cell proliferative diseases and conditions, and in particular, for use as antitumor agents.

Background of the Invention

[002] The report by Kupchan and coworkers in 1972 on the bioassay-guided isolation of the potent cytotoxic agent, maytansine from the Ethiopian shrub, *Maytenus serrata*, raised high hopes for its eventual use as a chemotherapeutic agent for the treatment of cancer. However, clinical trials with maytansine proved disappointing, showing no significant clinical benefits from its administration to human cancer patients. Nevertheless, because of their extremely high potency, maytansine and its congeners continue to command interest.

[003] It is accordingly a primary object of the invention to provide new maytansinoid analogs with improved antitumor activity.

SUMMARY OF THE INVENTION

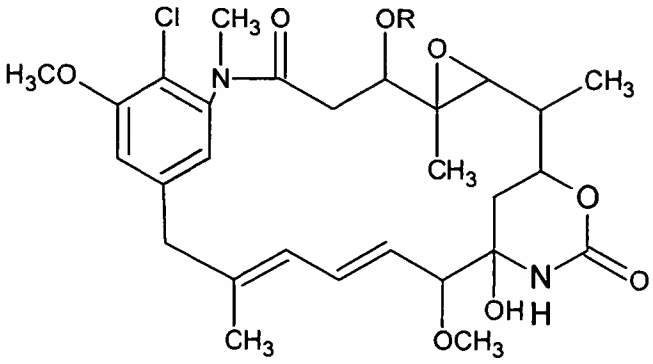
[004] Features and Advantages of the Invention

[005] The invention is advantageous in providing improved maytansinoid compounds with lower systemic toxicity, improved pharmacokinetic profile, and better clinical activity.

[006] Summary of the Invention

[007] In accordance with the invention, novel maytansinoid analogs are provided.

[008] The invention is directed to, for example, antitumor compounds having the following structure:



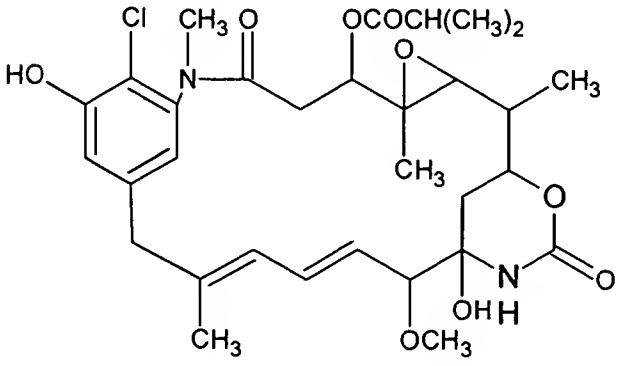
wherein R is chosen from:

Formula I: $CH_2COCH(CH_3)_2$,

Formula II: $CH_2CO(CH_2)_{16}CH_3$, and

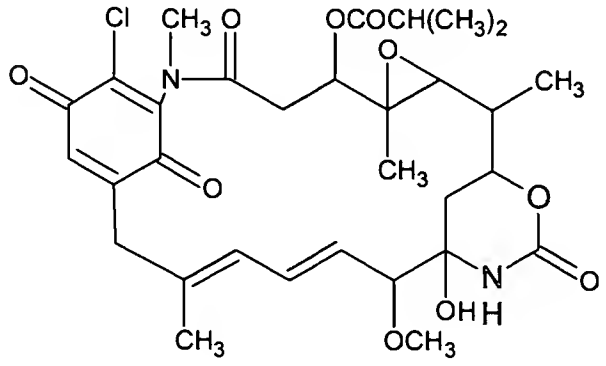
Formula III: $CH_2COCH(NH_2)Ph$.

[009] The present invention is also directed to antitumor compounds having structures similar to:



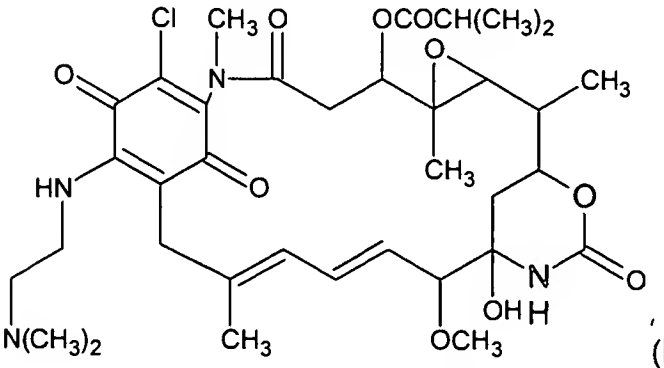
(Formula IV)

[010] The present invention is also directed to antitumor compounds having the following structure:



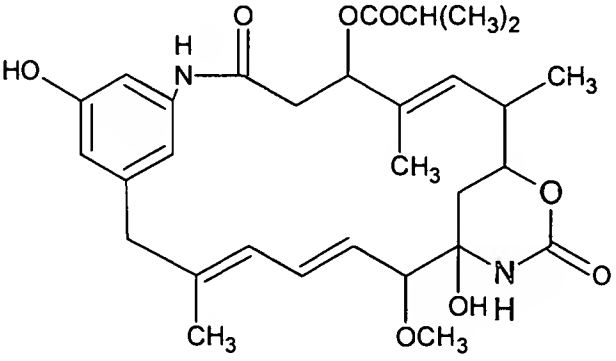
(Formula V).

[011] The present invention is also directed to antitumor compounds having the following structure:



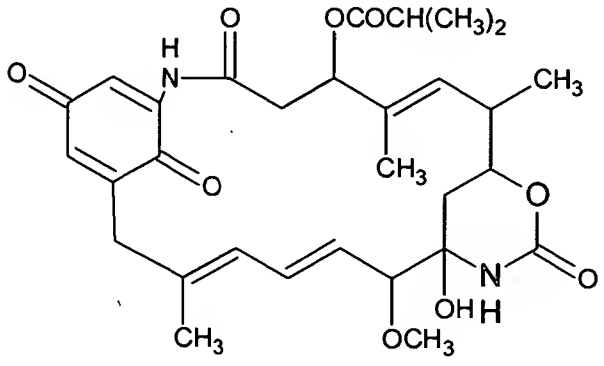
(Formula VI).

[012] The present invention is also directed to antitumor compounds having the following structure:



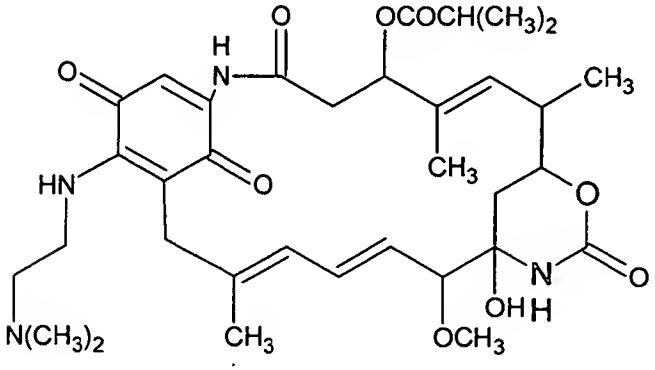
(Formula VII).

[013] The present invention is also directed to antitumor compounds having the following structure:



(Formula VIII).

[014] The present invention is also directed to antitumor compounds having the following structure:



(Formula IX).

[015] Additional features and advantages of the invention will be set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the invention. The features and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[016] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

DESCRIPTION OF THE EMBODIMENTS

[017] Reference will now be made in detail to specific embodiments (exemplary embodiments) of the invention. Throughout this disclosure, reference will be made to compounds according to the invention. Reference to such compounds, in the specification and claims, includes esters and salts of such compounds. Thus, even if not explicitly recited, such esters and salts are contemplated, and encompassed, by reference to the compounds themselves.

[018] As used herein, the term "hydrocarbyl" includes, but is not limited to, "aliphatic," "cycloaliphatic," and "aromatic" groups. Thus, hydrocarbyl groups include, but are not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, and alkaryl groups. Further, "hydrocarbyl" is understood to include both non-substituted hydrocarbyl groups, and substituted hydrocarbyl groups, with the latter referring to the hydrocarbon portion bearing additional substituents, besides carbon and hydrogen.

[019] The present invention is generally directed to novel compounds having structures related to the maytansinoid group, and in some cases, to the geldanamycin group, and to methods of use of these compounds in the treatment of cell proliferative diseases and conditions. Maytansinoids generally target tubulin, whereas geldanamycins generally target heat shock protein-90 (HSP-90).

Compounds of the present invention can target tubulin, HSP-90, or both, and can exhibit a cytotoxic effect through one or both of these mechanisms.

[020] The term "cell proliferative disease or condition" is meant to refer to any condition characterized by aberrant cell growth, preferably abnormally increased cellular proliferation. Examples of such cell proliferative diseases or conditions include, but are not limited to, cancer, restenosis, and psoriasis. In some embodiments, the invention provides a method for inhibiting neoplastic cell proliferation in an animal comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of a compound of the invention. Cancers treatable according to the invention include, but are not limited to, prostate cancer, lung cancer, acute leukemia, multiple myeloma, bladder carcinoma, renal carcinoma, breast carcinoma, colorectal carcinoma, neuroblastoma, or melanoma. Other diseases treatable with the present compounds include fungal infections or infestations; the present compounds can be used for any control of fungal growth.

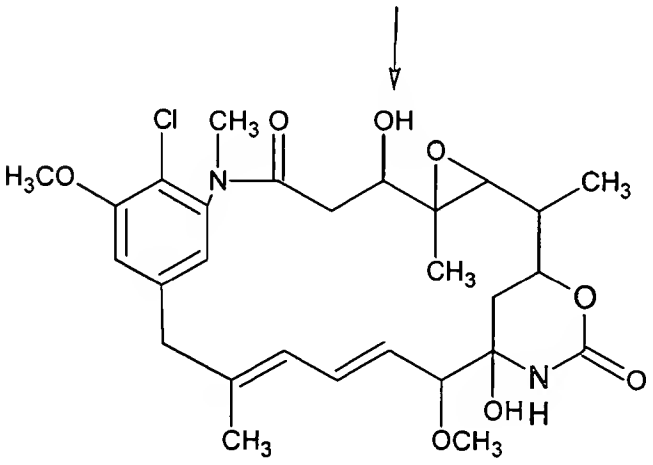
[021] Work with maytansine has been disappointing due to dose-limiting toxicity in humans. However, the fact that animal tests have proved effective suggests that the problem lies in differences in metabolism. Thus, without wishing to be bound by any particular theory, the present invention strives to improve clinical effects of these compounds by changing the manner in which they are metabolized. The effect is to produce compounds with reduced toxicity but better clinical efficacy.

[022] The compounds of the invention include, but are not limited to, two major groups. Group I includes non-hydrolyzable esters (analogs) of ansamitocin P-

3 (AP3) or maytansine. The ester moiety is believed to be important for tubulin binding and cytotoxicity. Although metabolism and pharmacokinetic studies are still in progress, it is clear that the ester is modified and is susceptible to decomposition with time.

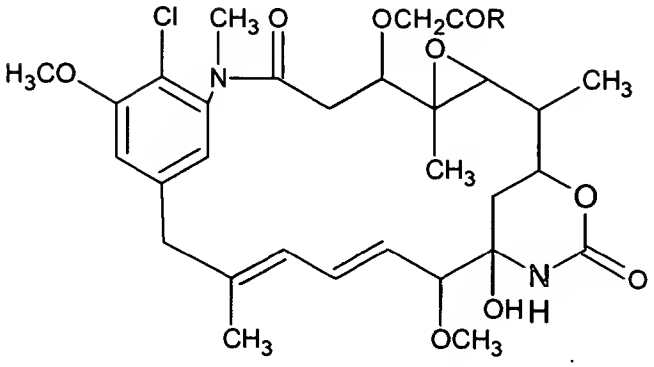
[023] The second major group of compounds include hybrid molecules that incorporate the potential to target both tubulin (a maytansinoid quality) and HSP-90 (a geldanamycin quality). These hybrid analogs can be generally referred to as "geldanamitocins."

[024] Group I compounds (e.g., the "non-hydrolyzable" analogs) can be synthesized by any method that will yield the compounds as described herein. One example of a method that can be used involves the reductive cleavage of ansamitocin P-3 to yield maytansinol:

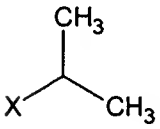


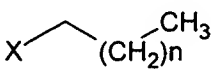
The arrow indicates the site of reaction.

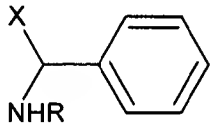
[025] Maytansinol is reacted (at the C(3) hydroxyl) with R-$COCH_2Cl$ to yield:



wherein R comprises any hydrocarbyl group. Other examples of R include but are not limited to:

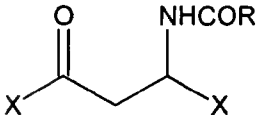


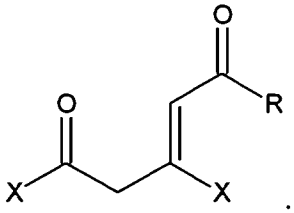




Where n is from 1 to about 20. (X refers to the remainder of the molecule.)

[026] If the reaction at the C(3) hydroxyl (shown by the arrow in the maytansinol structure above) is not feasible, then other targets can be designed, including, for example:





Again, the X refers to the remainder of the maytansinol structure and R is as described above.

[027] Compounds of Group II can be synthesized by transforming AP3 into 20-*O*-demethyl-AP3, for example, through use of *Bacillus megaterium* IFO 12108.

The demethyl-AP3 can then be oxidized to the quinone through numerous of reactions. The quinone can then be converted into the 17-DMAG analog by addition of, for example, 2-*N,N*-dimethylaminoethylamine.

[028] Other aspects of the invention relate to improving the ansamitocin production yield of *Actinosynnema pretiosum* by genetically manipulating the regulatory controls of ansamitocin biosynthesis and/or by gene shuffling.

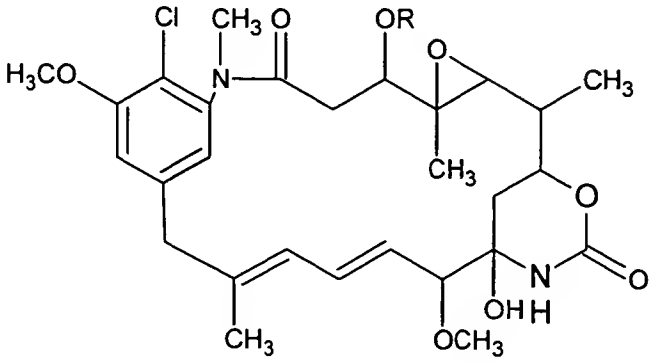
[029] A review of the maytansinoid compounds as anti-tumor agents is presented in "Recent Developments in the Maytansinoid Antitumor Agents," by Cassady et al., Chem. Pharm. Bull. 52(1): 1-26 (January 2004). The entire disclosure of the Cassady et al. review article is incorporated herein by reference.

[030] Examples

[031] Example 1: Non-Hydrolyzable Ester Analogs of AP3 and their Antitumor Activity

[032] Ansamitocin P-3 (AP3) is reduced with $Li(OMe)_3AlH$ to produce maytansinol. Chloromethylketone derivatives are prepared from isobutyric acid, hexadecanoic acid, and phenylglycine, respectively, by conversion to acid chloride (which may require N-protection in the case of phenylglycine), reaction with diazomethane, and reaction of the diazoketone with HCl.

[033] Maytansinol is reacted with the three chloroketones to produce the analogs of Formulas I, II, and III:



wherein R is chosen from:

Formula I: $CH_2COCH(CH_3)_2$,

Formula II: $CH_2CO(CH_2)_{16}CH_3$, and

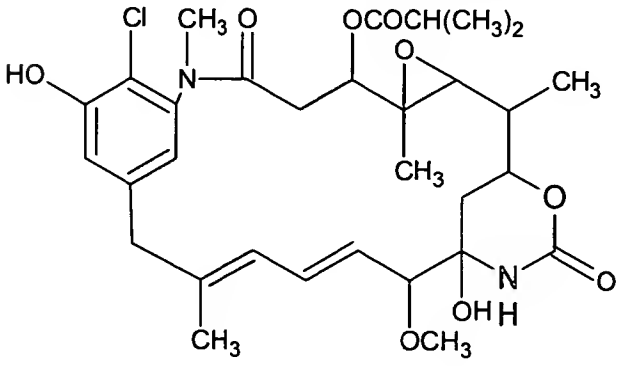
Formula III: $CH_2COCH(NH_2)Ph$.

[034] The three analogs are tested for cytotoxicity in appropriate cancer cell lines and in a tubulin binding assay.

[035] Example 2: Production of Maytansinoid-Geldanamycin Hybrid-Type Molecules

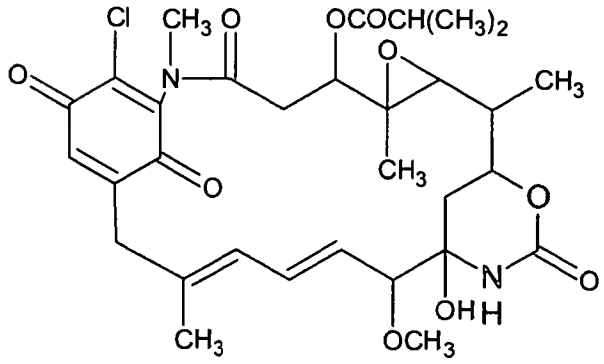
[036] In this Example, hybrid molecules are constructed that combine the mode of action of maytansinoids, i.e., inhibition of tubulin polymerization, with that of geldanamycin, i.e., inhibits heat shock protein 90 (HSP-90). In particular, the hybrid molecules retain the cyclic carbinolamide structure of the ansamitocins.

[037] Biotransformation of AP3 is carried out using *Bacillus megaterium* IFO 12108 to produce 20-*O*-demethyl-AP3 (this procedure is known in the art and has been described in detail elsewhere):



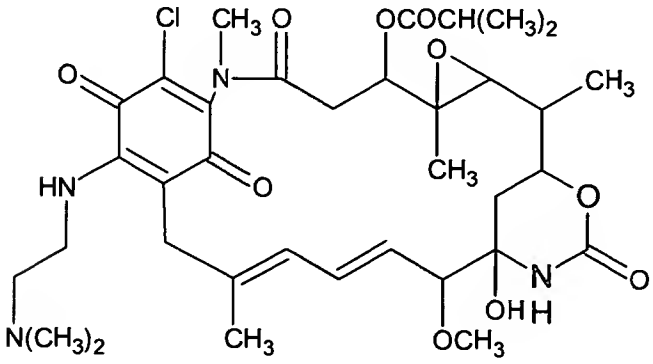
(Formula IV).

[038] The compound of Formula IV is then oxidized to yield the quinone:



(Formula V).

[039] The quinone (Formula V) is then converted into the 17-DMAG analog (Formula VI) by addition of 2-*N,N*-dimethylaminoethylamine.



(Formula VI).

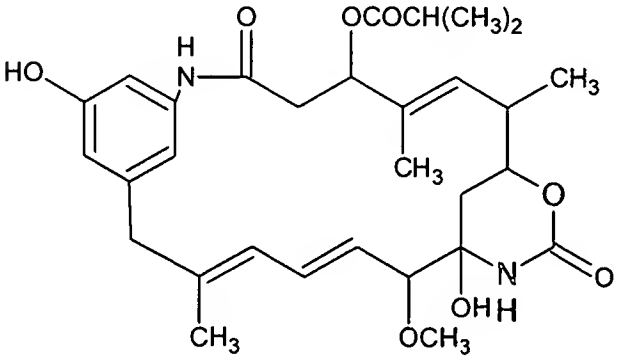
[040] The compounds of Formulas IV, V, and VI are then tested for tubulin binding and for HSP-90 binding, as well as for cytotoxicity.

[041] Example 3: Preparation and Testing of Additional Analogs

[042] This Example describes the preparation and testing of additional analogs.

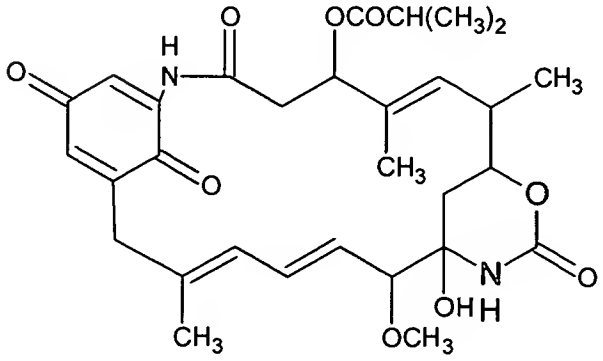
[043] A mutant of *Actinosynnema pretiosum* is engineered in which genes asm7, 10, 11, and 12 have been deleted. The genotype is then confirmed.

[044] The mutant is fermented and cultures are assayed for production of deschloro-20-*O*-demethyl-*N*-demethyl-desepoxy-AP3:



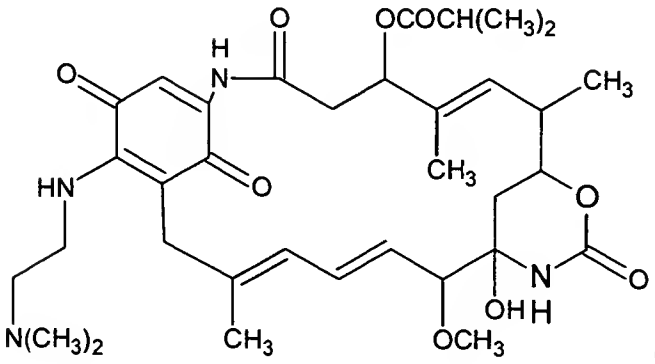
(Formula VII).

[045] The compound of Formula VII is oxidized to the quinone of Formula VIII:



(Formula VIII).

[046] The quinone (Formula VIII) is derivatized to yield the 17-DMAG analog:



(Formula IX).

[047] Compounds VII, VIII, and IX are tested for general cytotoxicity, for HSP-90 binding, and for tubulin binding.

[048] While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing from the spirit and scope of the invention. In addition, while the present invention has been described in connection with certain specific embodiments thereof, it is to be understood that this is by way of illustration and not by way of limitation and the scope of the invention is defined by the appended claims which should be construed as broadly as the prior art will permit.

[049] The disclosure of all patents, patent applications (and any patents which issue thereon, as well as any corresponding published foreign patent applications), and publications mentioned throughout this description are hereby incorporated by reference herein. It is expressly not admitted, however, that any of

the documents incorporated by reference herein teach or disclose the present invention.

[050] It should be understood that every maximum numerical limitation given throughout this specification will include every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[051] Except where otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

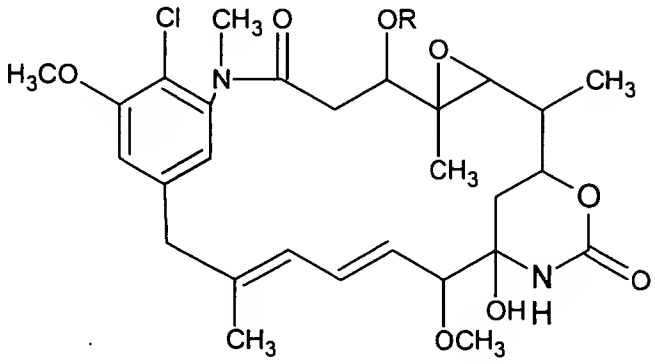
[052] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of the ordinary skill in the art to which this invention belongs. The terminology used in the description of

the invention herein is for describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

[053] The specification is most thoroughly understood in light of the teachings of the references cited within the specification, all of which are hereby incorporated by reference in their entirety. The embodiments within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan recognizes that many other embodiments are encompassed by the claimed invention and that it is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

WHAT IS CLAIMED IS:

1. A compound having the following structure:



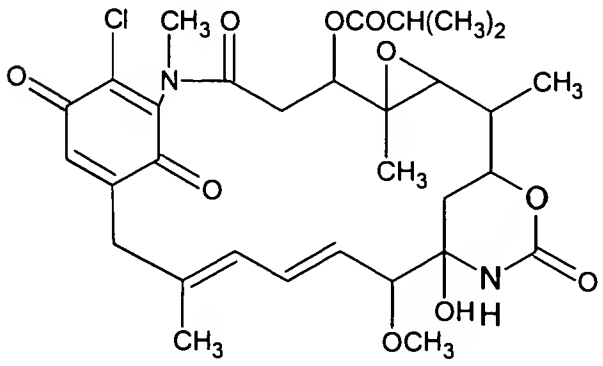
wherein R is chosen from:

I: $CH_2COCH(CH_3)_2$,

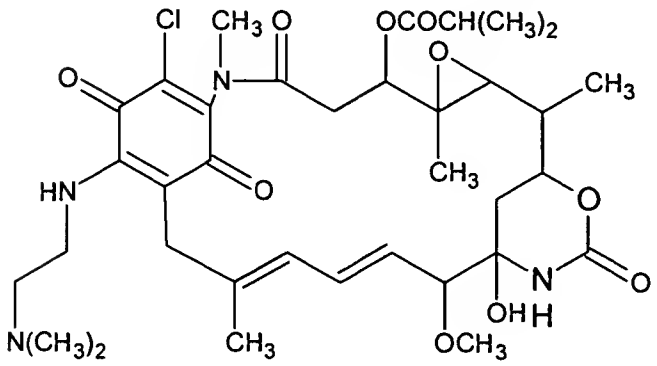
II: $CH_2CO(CH_2)_{16}CH_3$, and

III: $CH_2COCH(NH_2)Ph$.

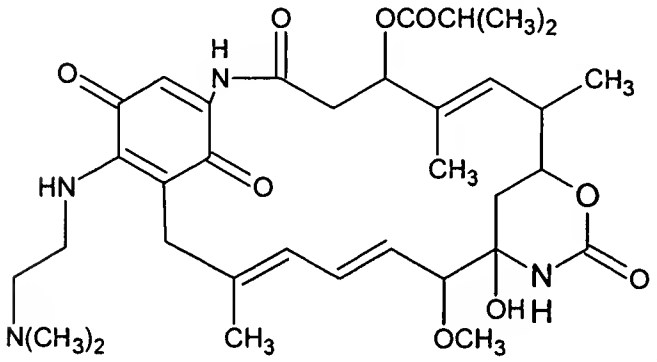
2. A compound having the following structure:



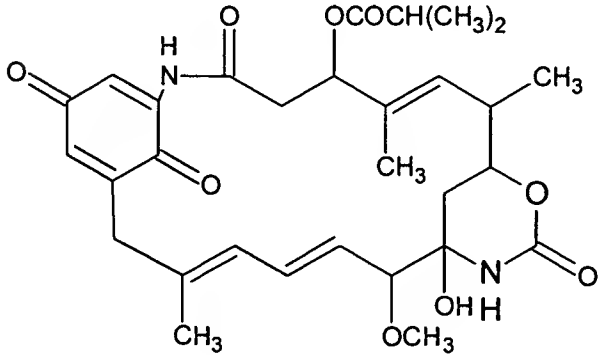
3. A compound having the following structure:



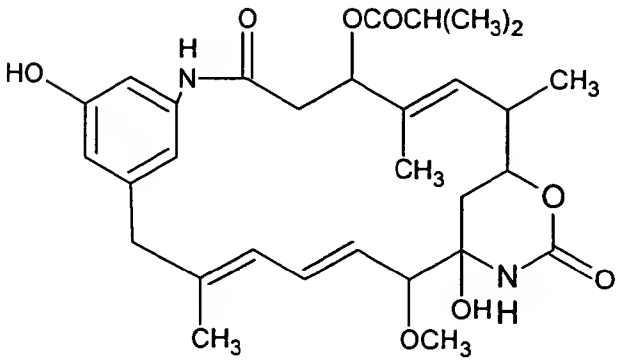
4. A compound having the following structure:



5. A compound having the following structure:



6. A compound having the following structure:



7. A method for treating a cell proliferative disease or condition comprising administering a therapeutically effective amount of at least one compound according to any one of claims 1-6.

8. A method of inhibiting fungal growth comprising administering a therapeutically effective amount of at least one compound according to any one of claims 1-6.

ABSTRACT OF THE DISCLOSURE

Ansamycin analogs, including maytansinoid analogs, and their use in treating cell proliferative diseases and conditions, and in particular, for use as antitumor agents.